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Positron Emission Tomography (PET) allows estrogen receptor-positive breast cancer to be diagnostically imaged using radiolabeled estrogens with the benefit of early detection, monitoring, and staging of breast cancer. In order to better target breast cancer, improved estrogen receptor imaging agents are sought.

Three new estrogen receptor ligands were synthesized with a methoxy substituted at the 16 position. These compounds were obtained by utilizing the chemistry of methyl hypofluorite (MeOF). Conditions for reacting methyl hypofluorite and steroids were optimized overcoming solubility and solvent problems resulting in decreased formation of side products.

Two of the three estradiol analogs were tested for their affinity to bind the estrogen receptor. The results predict these agents to be poor visualizers of breast tumors. The third estrogen, 16α-methoxyestradiol-17β, is currently being tested and based on its structure is predicted to be the superior imaging agent of the three. Synthesis of this third compound required several modifications leaving it the last to be tested. If the binding affinity of this compound is found to be favorable, carbon-11 radiolabeling studies will be initiated to further evaluate the biological effectiveness.

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Stephanie D. Jonson 6/28/97

PI - Signature Date

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New Approaches To The Labeling Of Estrogens Useful For PET

Introduction

Imaging estrogen receptors found in estrogen receptor-positive (ER+) breast cancer is made possible through the combined use of radiolabeled estrogens and Positron Emission Tomography (PET). This specific targeting allows non-invasive disease diagnosis and therapy monitoring. The advantages of diagnosing breast cancer at an early stage has spurred research toward the development of improved imaging agents.

Currently, the clinical radiopharmaceutical for breast cancer imagery is [^{18}F]-16α-fluoroestradiol-17β (FES).[5,12,13] Studies with FES display the ability of radiolabeled estrogens and PET to effectively diagnose breast cancer, however, several characteristics of this agent need improvement. This instigated the search for estrogens with higher binding affinity for the estrogen receptor, slower metabolism, and incorporation of a radionuclide with a shorter half-life than fluorine-18 (half-life = 110 m) to decrease the radiation dose given the patient. Substitution of carbon-11 (half-life = 20.4 m) for fluorine-18 was proposed to decrease the radiation dose given the patient, however, carbon-11 chemistry is limited to a small set of carbon-11 precursors restricting the synthesis of estrogen receptor ligands.[10] Therefore, new synthons capable of incorporating carbon-11 into molecules on a short time scale are sought to access estrogen receptor imaging with carbon-11 radioligands.

Methyl hypofluorite (CH_3OF) is one such new carbon-11 incorporating synthon.[11] Since the reported isolation and characterization of methyl hypofluorite (CH_3OF) studies have focused on its reactivity.[9] Methyl hypofluorite is generated from passing fluorine gas (20% in Ne) through methanol in acetonitrile at -40 °C and is reported as the only source of the novel electrophilic methoxylium ion species "CH_3O^+".[17] Various enol ethers were found to react with CH_3OF and rapidly form the corresponding α-methoxy ketone.[17]

Previously, compounds of this sort were chemically difficult to obtain requiring multi-step syntheses.[3,14] Applying the chemistry of methyl hypofluorite to the preparation of novel estrogen receptor ligands provides a means of rapid introduction of a methoxy functionality. This allows the biological evaluation of new estrogen derivitives to give useful structure-activity information about the estrogen receptor-ligand binding interaction.

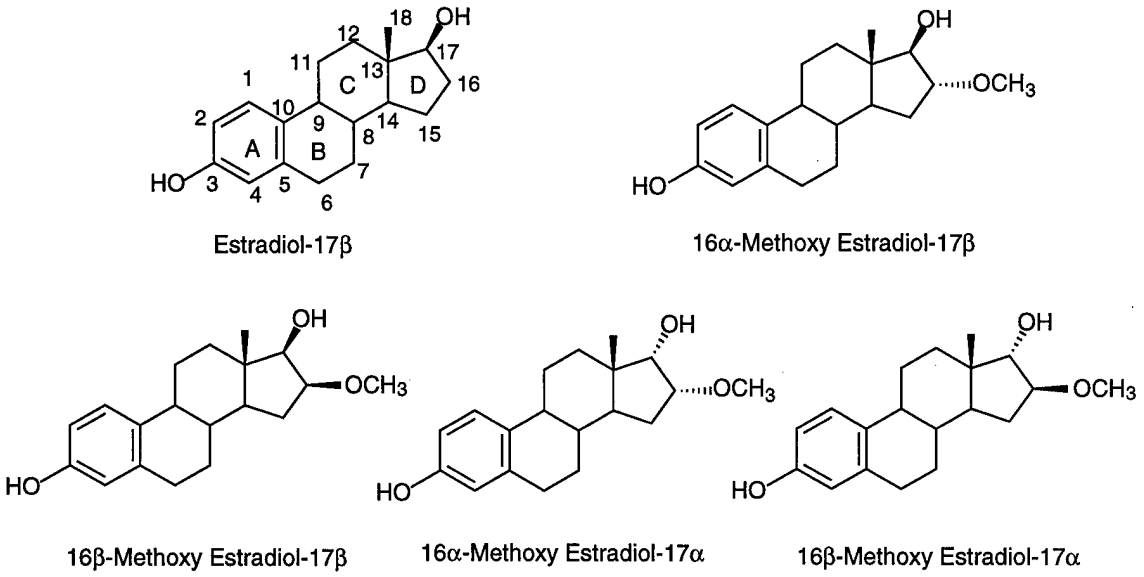


Figure 1.

The targeted compounds for synthesis with CH_3OF were the 16-methoxy estradiol stereoisomers. Four isomers are possible as the α- and β- orientation can differ at the 16 and 17 positions (Figure 1). It is known that estrogen receptor binding requires a ligand to have the 17β-OH orientation. Therefore, the two isomers with this orientation, 16α-methoxy estradiol-17β and 16β-methoxy estradiol-17β were desired over the two isomers with the 17α- configuration.[6] Previously, only halogen substitutions at the 16-position of estradiol were accessed. Substitutions of fluorine, bromine, and iodine were previously accomplished at both the 16α- and 16β-position and relative binding affinity (RBA)

determinations for the estrogen receptor (ER) revealed a preferrence for 16α- over 16β-substitution (Table 1).[6] This led to the prediction of 16α-methoxy estradiol-17β as the isomer with superior binding affinity for the ER. However, this prediction was contingent on the change from an electron-withdrawing halogen to an electron-donating methoxy not disrupting the receptor-ligand binding interaction.

Table 1. Relative binding affinities (RBA) of estrogen receptor ligands substituted at the 16-position.

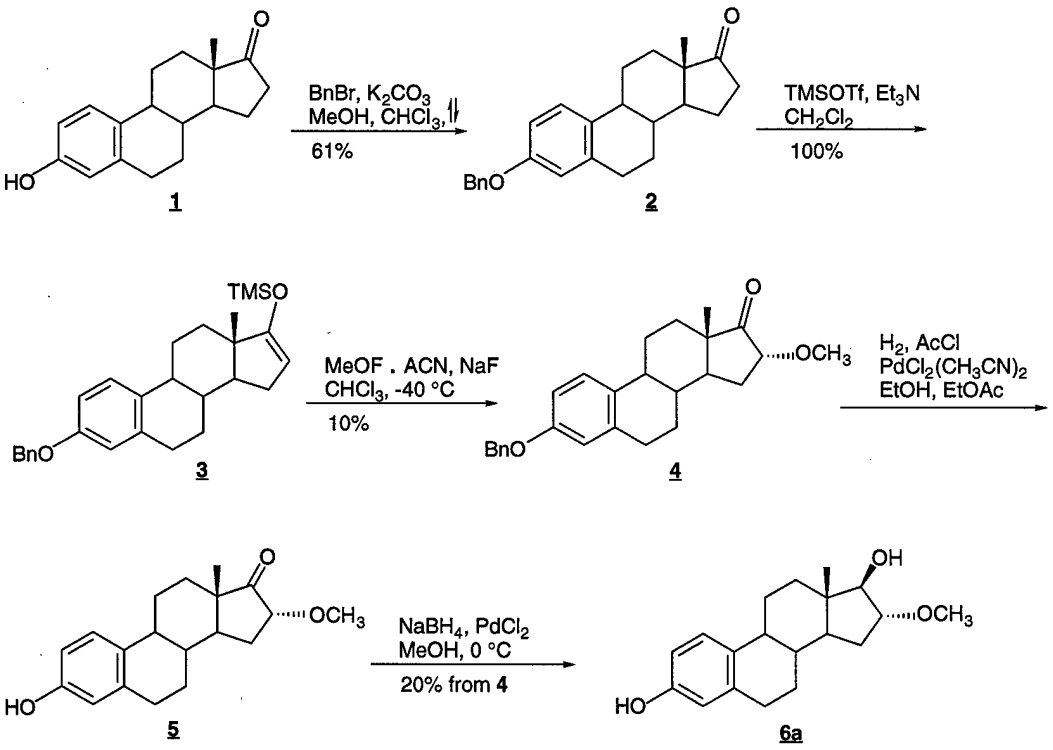
Compound	16α	16β	RBA (0 °C)
Estradiol (ES)	--	--	100
Estriol	OH	--	20
16α-FES	F	--	76
16β-FES	--	F	37
16α-Chloro Estradiol	Cl	--	100
16α-Bromo Estradiol	Br	--	129
16β-Bromo Estradiol	--	Br	5.2
16α-Iodo Estradiol	I	--	93
16β-Iodo Estradiol	--	I	81

Combining the chemistry of CH_3OF with steroids required the transfer of reaction conditions derived from simple substrates such as enol acetate of 1-indanone[17] to the more chemically complex steroidal substrates. Reaction conditions were optimized for the steroid system to minimize solubility problems and side product formation. The methoxy substituent was introduced into the estrogen by reacting the 17-trimethylsilyl enol ether-3-trifloxy (or benzyloxy) estrone with methyl hypofluorite. Deprotection and reduction conditions were varied to yield the various methoxy estradiol stereoisomers. HPLC purification was required to separate the isomers which were characterized by ^1H NMR and HMQC, HMQC-TOCSY, and NOESY two-dimensional NMR experiments.

Results and Discussion

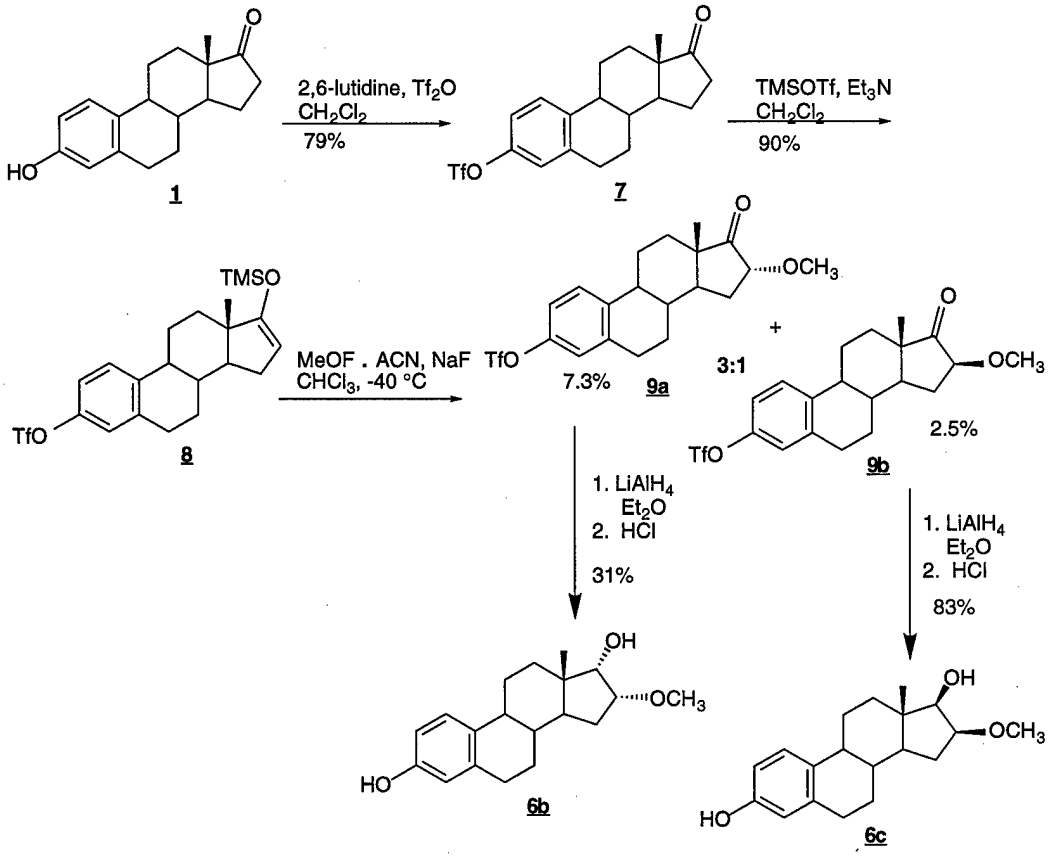
Chemical Syntheses

Our synthetic approach to 16α-methoxy estradiol-17β (**6a**) involved reacting the trimethylsilyl enol ether of 3-benzyl estrone (**3**) with CH_3OF under optimized reaction conditions (Scheme 1). This reaction selectively yielded the 16α-methoxy isomer with minimal or no formation of a 16β-methoxy product as assessed by ^1H NMR by identifying the -OCH_3 shift at ca. 3.5 ppm. The electron-donating benzyl group on the A-ring affected the reactivity of the D-ring silyl enol ether of the estrogen. While ^1H NMR showed the desired methoxy ketone in 23% yield, column purification led to only a 10% overall yield for this reaction.



Scheme 1. Synthesis of 16α-Methoxyestradiol-17β.

Reacting CH_3OF with the silyl enol ether of 3-trifloxy estrone (**8**) yielded an isomeric mixture of 16α- and 16β-methoxy-3-OTf-estrone (**9a** and **9b**) in a 3:1 ratio, respectively (Scheme 2). The electron-withdrawing A-ring triflate increased the reactivity of the D-ring increasing the yield of methoxy products to 25-37% identified by ^1H NMR of the crude reaction mixture. The increased reactivity led to formation of the 16β–isomer requiring dual purification (silica gravity column chromatography; HPLC) to separate the 16α- from the 16β- isomer. This decreased the combined yield of isolated isomers to 10%.



Scheme 2. Synthesis of 16α-methoxyestradiol-17α and 16β-methoxyestradiol-17β.

Initial MeOF reactions involved direct addition of the enol ether substrate dissolved in CH_2Cl_2 to the CH_3OF•ACN at -40 °C. This yielded a crude mixture of ca. 8 products detected by TLC with only minor formation of the desired product. A precipitate formed

upon substrate addition to the CH_3OF flask influencing the low yield of desired product. The substrate was determined to be insoluble in this composition of ACN and CH_2Cl_2 at -40 °C causing precipitation. Conditions for substrate addition to CH_3OF•ACN were configured to maintain solubility of the enol ether while maintaining the reactivity of CH_3OF. The desired product yield was further increased by changing the substrate solvent to radical scavenging $CHCl_3$.

Side product formation, presumably from reacting with HF formed during the generation of CH_3OF, was decreased by the addition of oven dried NaF to the CH_3OF•ACN immediately prior to transferring this solution to the CH_2Cl_2 dissolved substrate. Addition of NaF acts as a fluoride ion acceptor to decrease the acidity of HF through the formation of the HF_2^- ion which seqesters the ability of HF to react with the substrate.[4]

Reduction and deprotection of 16α-methoxy-3-OTf-estrone (**9a**) with $LiAlH_4$ led to selective formation of the 16α-methoxy estradiol-17α (**6b**). This was unexpected as $LiAlH_4$ was used to reduce and deprotect 16α-fluoro-3-OTf-estrone leading to the deprotected 17β-OH and 17α-OH estradiols in a 3:1 ratio.[8] The 16β-methoxy-3-OTf-estrone (**9b**) was reduced with $LiAlH_4$ to give the 16β-methoxy estradiol-17β (**6c**). This configuration was expected for the hydride can only attack from the α-face of the steroid as the 18β-methyl and 16β-methoxy are blocking the β-face. The combined reduction/deprotection step with $LiAlH_4$ was advantageous for past steroid syntheses, however, for this study it did not yield the desired 17β-OH configuration with the 16α-methoxy isomer (**9a**). Unexpected 17α- and 17β-OH ratios were also seen with the $LiAlH_4$ reduction/deprotection of 11β-ethyl-16β-fluoro-3-OTf-estrone. With the β-face being blocked by both the 11β–ethyl, 16β–fluoro, and 18β-methyl substituents, the hydride attack from the unhindered α-face was expected, however, the attack from the shielded β-face prevailed by 1.6:1.[16]

Choice of reagent and reaction order determined which stereoisomer was preferrentially formed. Desiring to selectively reduce **6a** to the 17β-OH, a method using sodium borohydride ($NaBH_4$) in the presence of palladium chloride was applied as it is cited as selectively reducing 16α-hydroxyestrone and 16α-acetoxyestrone to the corresponding 17β-estradiols.[15] Direct application of this chemistry to 16α-methoxy-3-OTf-estrone (**9a**) proved unacceptable. Reduction of the trifloxy protected estrone in the presence of palladium led to 16α-methoxy-17β-estrol formation. To preserve the hydroxy at the 3-position, deprotection of the triflate needed to preceed ketone reduction. Deprotection with KOH/methanol at 60 °C sucessfully deprotected the triflate, however, base racemized the 16α-methoxy favoring 16β-methoxy formation 2:1. Stereoselective reduction in the presence of palladium chloride required prior deprotection in the absence of base. This was accomplished by protecting the 3-OH as a benzyl ether allowing rapid debenzylation by hydrogenolysis prior to $NaBH_4$ reduction in the presence of palladium.

2-D NMR Characterization

^1H NMR steroid peaks are difficult to assign due to severe overlap of resonances. To confirm the isomeric configurations of the methoxy estradiol compounds, two-dimensional (2-D) correlated NMR techniques were instituted. COSY-like (H, H) methods of analysis generally fail for steroids as the ^1H dispersion is poor. Good ^{13}C dispersion seen with steroids makes identification techniques like HMQC-TOCSY (heteronuclear multiple quantum coherence - total correlation spectroscopy) useful as steroids have carbons with attached protons on the B, C, and D rings. TOCSY provides information in a two-dimensional format indicating the correlations between protons belonging to a common spin system.[1] HMQC experiments correlating ^1H-^{13}C one-bond coupling combined with the extended coupling identified by HMQC-TOCSY allows assignment of the steroid skeleton. The HMQC and HMQC-TOCSY assignments for compounds **6a**, **6b**, and **6c** are listed in Tables 2, 3, and 4 respectively.

Table 2: HMQC and HMQC-TOCSY assignments for 16α-Methoxy Estradiol-17β (**6a**).

16α-OMe-E2-17β Assignment No.[a]	HMQC ^{1}H Chemical Shift (ppm)	HMQC ^{13}C Chemical Shift (ppm)	HMQC-TOCSY Chemical Shift: ^{13}C to ^{1}H Shift of HMQC (ppm)
17	3.64	88.2	----
16	3.70	88.0	30.9
15	1.72	30.9	48.4; 88.0
14	1.50	48.4	30.9; 38.8
12	1.35; 1.91	37.0	26.5
11	1.47; 2.29	26.5	37.0
9	2.22	44.2	26.5; 38.8
8	1.43	38.8	27.6; 44.2; 48.4
7	1.36; 1.85	27.6	29.9; 38.8
6	2.82	29.9	27.6
18	0.81	13.0	---
OMe	3.39	57.9	---

[a]Corresponds to the steroidal numbering system.

Table 3: HMQC and HMQC-TOCSY assignments for 16α-Methoxy Estradiol-17α (**6b**).

16α-OMe-E2-17α Assignment No.[a]	HMQC ^{1}H Chemical Shift (ppm)	HMQC ^{13}C Chemical Shift (ppm)	HMQC-TOCSY Chemical Shift: ^{13}C to ^{1}H Shift of HMQC (ppm)
17	3.76	77.3	81.5
16	4.00	81.5	31.6; 77.3
15	1.72	31.6	46.6; 81.5
14	1.88	46.6	31.6; 39.0
12	1.63; 1.96	31.6	26.1
11	1.52; 2.30	26.1	31.6; 44.0
9	2.24	44.0	26.1; 39.0
8	1.35	39.0	28.3; 44.0; 46.6
7	1.40; 1.84	28.3	30.0; 39.0*
6	2.82	30.0	28.3
18	0.71	17.0	---
OMe	3.40	58.0	---

[a]Corresponds to the steroidal numbering system.
*Not seen for ^{1}H = 1.84.

Table 4: HMQC and HMQC-TOCSY assignments for 16β-Methoxy Estradiol-17β (**6c**).

16β-OMe-E2-17β Assignment No.[a]	HMQC ^{1}H Chemical Shift (ppm)	HMQC ^{13}C Chemical Shift (ppm)	HMQC-TOCSY Chemical Shift: ^{13}C to ^{1}H Shift of HMQC (ppm)
17	3.49	79.0	81.1
16	3.74	81.1	32.1; 79.0
15	1.73	32.1	46.5; 81.1
14	1.80	46.5	32.1; 38.2
12	1.62; 1.96	31.9	26.1
11	1.50; 2.35	26.1	31.9; 44.1
9	2.25	44.1	26.1; 38.2
8	1.35	38.2	27.4; 44.1; 46.5
7	1.42; 1.87	27.4	30.6; 38.2*
6	2.82	30.6	27.4
18	0.79	**	---
OMe	3.37	57.6	---

[a]Corresponds to the steroidal numbering system.
*Not seen for ^{1}H = 1.87.
**Due to an impurity, the assignment was indistinguishable from 14.1 or 11.7 ppm.

Confirmation of the stereochemistry at the 16- and 17-position was obtained by a NOESY (nuclear Overhauser effect spectroscopy) experiment which looked at dipole-dipole interactions through space (Table 5). The combination of these techniques was essential for assignment of each isomer's D-ring stereochemistry.

Table 5. NOESY assignments represented as the average relative volume to confirm the stereochemistry of 16-Methoxy Estradiol isomers.

Interacting H Pairs	NOE 16α-OMe-E2-17β (**6a**)	NOE 16α-OMe-E2-17α (**6b**)	NOE 16β-OMe-E2-17β (**6c**)
H_{17} - H_{16}	0.271	0.825	0.271
H_{17} - H_{14}	1.852	0.058	---
H_{17} - OMe	0.376	0.148	**
H_{16} - OMe	1.184	0.828	0.440
18-CH_3 - H_{16}	1.160	0.808	0.063
18-CH_3 - H_{17}	---	0.833	0.112

---A missing value represents that an NOE was not seen for this interaction.
**Represents an obscured interaction by either a cross-peak or an artifact.

Relative Binding Affinities

The relative binding affinities (RBA) of the 16-methoxy estradiols for the estrogen receptor were determined by a competitive radiometric binding assay using lamb uterine estrogen receptor.[7] The highest RBA for the methoxy estradiol series was 2.3 for **6c** while the RBA for **6a** and **6b** was 1.5 and 0.5, respectively. The isomers of 16-methoxy estradiol all displayed low binding affinity for the ER compared to estradiol and 16-halogenated estradiols (Table 1). Halogens substituted at the 16α-position retain binding affinity suggesting that the receptor-ligand interaction is maintained with electon-withdrawing groups. The electron-donating methoxy may have disrupted this interaction leading to the unfavorable ligand-receptor association.

The size of the methoxy substituent may be an additional contributor to the low RBA. The space occupied by the methoxy is greater than that of iodine, the largest substituted halogen (Table 6). Comparing the relative binding affinities for the halogens shows a decrease in RBA between chlorine and iodine. If size is a problem, it is hypothesized that the RBA decreases as the size of a substituted group at the 16-position increases beyond 2 Å.

Table 6. Correlation of volume occupied at the 16-position of estradiol to the estrogen receptor's RBA.

Substitution at the 16α-Position	Size of Group (Å)	RBA for the ER
F	1.38	76
Br	1.94	100
Cl	1.76	129
I	2.08	93
OMe	2.69	1.5

Conclusion

Two different synthetic routes were required to obtain three isolated isomers of 16-methoxy estradiol. Methyl hypofluorite incorporated the methoxy at the 16-position of the steroid skeleton allowing evaluation of this ligand's ability to bind the ER. This study transferred the chemistry of CH_3OF from simple molecules to those of a more complex nature. The chemistry of CH_3OF was useful for the synthesis of methoxy substituted estrogens and will be applied to the compounding of a future steroidal target compound. Methyl hypofluorite will introduce a methoxy substitution at the 15-position of estradiol. It is unknown how a substitution at this position will affect binding affinity to the estrogen receptor.

Two-dimensional correlative NMR techniques were instrumental in the characterization of these isomers. The stereochemistry of the isomers was confirmed by identification of dipole-dipole interactions with NOESY. HMQC and HMQC-TOCSY provided a solid means of mapping the steroid structure through analysis of ^{1}H-^{13}C coupling. These NMR techniques would be advantagous for further steroid characterization.

This series of compounds was investigated in the pursuit of new estrogen receptor breast cancer imaging agents. The tolerance of the estrogen receptor for substituted estrogen derivatives was futher mapped. A methoxy substitution at the 16-position of estradiol decreased the binding to the estrogen receptor due to steric and/or electronic effects. The low binding-affinities of the methoxy estradiols predict these compounds to be poor visualizers of the estrogen receptor, therefore, unsuitable as estrogen receptor imaging agents. Compounds with low affinity for the receptor will not selectively accumulate at the site of interest. Binding to the receptor at the target site is an important requirement for radiopharmaceuticals. Non-selective distribution would lead to an undesired radiation dose at non-target sites. These compounds will not be radiolabeled for further evaluation due to their unfavorable binding affinities. With a better understanding of the requirements for an

agent to bind the estrogen receptor, the engineering of superior breast cancer imaging agents can advance.

Comments

Much of this pre-doctoral research project has focused on the optimization , synthesis, characterization and evaluation of the three isomers of 16-methoxy estradiol. This took a high level of effort to achieve these target compounds that had not been previously prepared. Although these compounds turned out to be not useful, the information obtained will benefit several areas of chemistry and medicine.

Experimental Section

General. All commercial reagents were used as received from the suppliers unless otherwise noted. HPLC solvents were Optima grade. Fluorine (20% in Ne) was purchased from Acetylene Gas (St. Louis, MO). Due to the strong oxidizing and corrosive nature of fluorine, appropriate laboratory equipment should be instituted.[18] 2,6-Lutidine was distilled from barium oxide and stored over molecular sieves. Methylene chloride (CH_2Cl_2) and triethylamine (TEA) were distilled from calcium hydride (CaH_2). Column chromatography was performed using silica gel (60 Å, 230-400 mesh) or basic alumina (40 μm). TLC was performed on UV active 250 μm silica plates visualized with phosphomolybdic acid or potassium permanganate. Melting points are uncorrected. ^{1}H and ^{19}F NMR spectra were obtained on a Varian Associates, Inc., Gemini spectrometer at 300 (or 500 MHz) and 282 MHz, respectively. Chemical shifts for ^{1}H and ^{13}C are referenced to internal tetramethylsilane (δ scale). ^{19}F chemical shifts are reported in ppm upfield from internal $CFCl_3$ (ϕ scale). Microanalyses were performed by Galbraith Laboratories Inc. 3-[[(Trifluoromethyl)sulfonyl]oxy]estra-1,3,5(10)-trien-17-one (**7**) was prepared according to the literature.[8] General work-up of organic solutions included drying over $MgSO_4$, filtering, and removing solvent under reduced pressure.

3-(Benzyloxy)estra-1,3,5(10)-trien-17-one (2). A solution of **1** (1.2 g, 4.44 mmol) and BnBr (1.06 ml, 8.88 mmol) were added to a mixture of 50 ml $CHCl_3$, 25 ml MeOH, and K_2CO_3 (1.23 g, 8.88 mmol) that had refluxed under N_2 for 15 min. The reaction was refluxed for 21 hr, cooled to rt, filtered, and filtrate concentrated under reduced pressue. Residue was dissolved in CH_2Cl_2, washed with 1x100 ml 1 N HCl, followed by general work-up. Recrystallization from MeOH yielded **2** as a white solid (0.978 g, 61%). mp 126-128 °C. ^{1}H NMR ($CDCl_3$): δ 0.91 (s, 3H, 18-CH_3), 1.30-2.60 (m, 13H), 2.90 (m, 2H), 5.04 (s, 2H, $PhCH_2OAr$), 6.74 (d, J = 2.7, 1H), 6.79 (dd, J = 8.6, 2.7, 1H), 7.21 (d, J = 8.7, 1H), 7.32-7.45 (m, 5H). HRMS calcd for $C_{25}H_{28}O_2$ (M^+) 360.2089, found 360.2081.

17-(Trimethylsilyl)oxy-3-(benzyloxy)estra-1,3,5(10),16-tetraene (3). To a solution of benzy ketone **2** (0.770 g, 2.14 mmol) in 15 mL CH_2Cl_2 under N_2 was added Et_3N (1.55 mL, 11.1 mmol, 5.2 eq). The solution was stirred for 20 m prior to addition of TMSOTf (1.24 mL, 8.88 mmol, 4 eq) followed by 30 m of stirring. Reaction was deposited directly onto a basic alumina column and eluted with 25% CH_2Cl_2, 75% hexane, 1% Et_3N, followed by general work-up. Product coeluted with unreacted starting material. Purification by basic alumina flash column chromatography (20% EtOAc, 80% hexane) yielded **3** as a white solid(0.92g, 100%). mp 105-107 °C. ^{1}H NMR ($CDCl_3$): δ 0.22 (s, 3H); 0.86 (s, 3H); 1.39-2.4 (m, 11H); 2.85-2.91 (m, 2H); 4.52 (m, 1H); 5.03 (s, 2H, $PhCH_2OAr$); 6.73 (d, J = 2.7, 1H); 6.78 (dd, J = 8.4, 2.7, 1H); 7.19 (d, J = 8.7, 1H); 7.31-7.45 (m, 5H). HRMS calcd for $C_{28}H_{36}O_2Si$ (M^+) 432.2485, found 432.2492.

17-(Trimethylsilyl)oxy-3-[[(trifluoromethyl)sulfonyl]oxy]estra-1,3,5(10),16-tetraene (8). Procedure A (adapted from Cazeau[2]). To a flask containing trifyl ketone **7** under N_2(0.514 g, 1.28 mmol), Et_3N (221 μL, 1.59 mmol, 1.24

eq) was added followed by TMSCl (201 μL, 1.59 mmol, 1.24 eq). The resulting white slurry was stirred while NaI (0.238 g, 1.59 mmol, 1.24 eq) in anhydrous ACN (1.6 mL) was added dropwise. Cold hexane and ice water were added after the solution was stirred at rt for ca. 66 h. After decantation, the aqueous layer was washed with hexane and the combined organic extracts were washed thrice with cold saturated sodium bicarbonate followed by general work-up. Purification by silica flash column chromatography (10% EtOAc, 90% hexane) yielded **8** as a white solid (0.304 g, 50%).

Procedure B. To a solution of trifyl ketone **7** (2.53 g, 6.29 mmol) in 40 mL CH_2Cl_2 under N_2 was added Et_3N (1.76 mL, 12.58 mmol, 2 eq). After the solution was stirred for 20 m and cooled to 0 °C, TMSOTf (2.44 mL, 12.58 mmol, 2 eq) was added. Cold bath was removed to allow the reaction to proceed at rt. Reaction was monitored by TLC (23% EtOAc, 77% hexane) and additional Et_3N (2.0 mL) and TMSOTf (1.5 mL) were added to maximize the yield of the enoxy silane over a reaction time of 3 h. Reaction mixture was deposited directly on a basic alumina plug and eluted with 25% CH_2Cl_2, 75% hexane, 1% Et_3N. Solvent removed under reduced pressure to afford the desired enoxy silane (2.70 g, 90%). mp 84-88 °C. ^{1}H NMR ($CDCl_3$): δ 0.21 (s, 9H); 0.88 (s, 3H); 0.97-2.10 (m, 11H); 2.35-2.40 (m, 2H); 4.55 (m, 1H); 6.72-6.82 (m, 3H). Anal. Calcd for $C_{22}H_{29}O_4F_3SSi$: C, 55.68; H, 6.16. Found: C, 56.12; H, 6.34.

General Procedure For Methyl Hypofluorite (CH_3OF) Reactions. To a N_2 swept flask were added 48 mL anhydrous ACN and 2 mL anhydrous MeOH and cooled to -40 °C in a dry ice/acetonitrile bath. Nitrogen flow was removed and F_2 (20% in Ne) was bubbled through the solution for 35 m. An aliquot (0.5 mL) was removed and added to a flask containing 25 mL H_2O and KF. Titration of the solution with $Na_2S_2O_4$ (equivalence point color change: yellow to colorless) determined the concentration of CH_3OF. The desired substrate was dissolved in $CHCl_3$ (10 mL) and cooled to 0 °C. NaF (30 mg) was added to the solution of CH_3OF and swirled for 30 sec before the CH_3OF was quickly

poured into the substrate flask. The reaction was stirred at 0 °C for 5 m followed by warming to rt over 40 m. Reaction was quenched by addition to saturated $NaHCO_3$ (250 mL) with stirring. Separation of the aqueous phase was following by washing the aqueous extract thrice with $CHCl_3$. The combined organic extracts were washed thrice with brine followed by general work-up.

16α-Methoxy-3-(benzyloxy)estra-1,3,5(10)-triene-17-one (4). The general procedure was followed to generate 6.90 mmol CH_3OF (0.139 M) to react with **3** (570 mg, 1.32 mmol). Purification by gravity silica column chromotography (30% hexane; 70% CH_2Cl_2) afforded **4** as a white solid (52 mg, 10%). ^1H NMR ($CDCl_3$): δ 0.88 (s, 3H, 18-CH_3); 1.26-2.10 (m, 11H); 2.85-2.95 (m, 2H); 3.52 (s, 3H, -OCH_3); 3.97 (d, J = 7.5, 1H); 5.03 (s, 2H); 6.70-6.81 (m, 2H); 7.19 (d, J = 8.7, 1H), 7.27-7.44 (m, 5H).

16α-Methoxy-3-[[(trifluoromethyl)sulfonyl]oxy]estra-1,3,5(10)-triene-17-one (9a). The above procedure was followed to generate 5.11 mmol CH_3OF (0.105 M) to react with **8** (330 mg, 0.695 mmol). Purification by silica flash column chromatography (15% EtOAc, 85% hexane) followed by semi-preparative normal phase HPLC (6% 1:19 isopropanol:CH_2Cl_2, 94% hexane) afforded **9a** as a white solid (36 mg, 12%). ^1H NMR ($CDCl_3$): δ 0.96 (s, 3H, 18-CH_3); 1.25-2.45 (m, 11H); 2.90-2.98 (m, 2H); 3.53 (s, 3H, -OCH_3); 3.98 (d, J = 7.2 Hz, 1H, 16-H); 6.98-7.06 (m, 2H); 7.34 (d, J = 8.5, 1H). HRMS calculated for $C_{20}H_{23}O_5F_3S$ $(M+H)^+$ 433.1296, found 433.1300.

16β-Methoxy-3-[[(trifluoromethyl)sulfonyl]oxy]estra-1,3,5(10)-triene-17-one (9b). The above procedure was followed to generate 5.11 mmol CH_3OF (0.105 M) to react with **8** (330 mg, 0.695 mmol). Purification by silica flash column chromatography (15% EtOAc, 85% hexane) followed by semi-preparative normal phase HPLC (6% 1:19 isopropanol:CH_2Cl_2, 94% hexane) afforded **9b** as a white solid (12 mg, 4%). ^1H NMR

($CDCl_3$): δ 1.00 (s, 3H, 18-CH_3); 1.22-2.58 (m, 11H); 2.93-2.98 (m, 2H); 3.54 (s, 3H, -OCH_3); 3.67 (t, J = 8.2 Hz, 1H, 16-H); 7.00-7.05 (m, 2H); 7.34 (d, J = 8.2, 1H). HRMS calculated for $C_{20}H_{23}O_5F_3S$ (M^+) 432.1218, found 432.1208.

16α-Methoxy-estra-1,3,5(10)-triene-3-ol-17-one (5). Benzyl ester **4** (17.3 mg, 0.045 mmol) was dissolved in 1 ml EtOAc and a suspension of 4 mg $PdCl_2(CH_3CH)_2$ and 8 µl EtOH was added. The reaction mixture was stirred under H_2 for 25 min, progressing through a color change from yellow to clear and colorless. The reaction mixture was diluted with CH_2Cl_2, filtered, and concentrated under reduced pressure. Crude reaction was redissolved in 1 ml EtOAc and passed through a silica plug eluted with 1:1 EtOAc:hexane. Procedure was repeated twice with **4**. Pooled reactions were not further purified. ^1H NMR showed complete deprotection. ^1H NMR ($CDCl_3$): δ 0.94 (s, 3H, 18-CH_3); 1.95-2.40 (m, 11H); 2.85 (m, 2H); 3.52 (s, 3H, -OCH_3); 3.98 (d, J = 7.4, 1H); 5.05 (b, < 1H, OH); 6.57-6.65 (m, 2H); 7.13 (d, J = 8.1, 1H).

16α-Methoxy-estra-1,3,5(10)-triene-3,17β-diol (6a). To a solution of **5** (13 mg, 0.0433 mmol) in 2 ml anhydrous MeOH was added $PdCl_2$ (15 mg, 0.087 mmol). While stirring under N_2, the reaction was cooled to 0 °C and $NaBH_4$ was added (9.8 mg, 0.260 mmol) and the reaction was stirred for 4 h. Reaction was filtered into 8 ml 5% HOAc followed by the addition of EtOAc and 1 M $NaHCO_3$. Following separation of layers, aqueous fraction was washed 3x15 ml EtOAc. Combined organic fractions were washed 3x30 ml H_2O followed by general work-up. Procedure was repeated twice with **5** and the crude reactions were pooled prior to semi-preparative silica HPLC purification (30% 1:19 isopropanol:CH_2Cl_2, 70% hexane) which yielded the methoxy estradiol **6a** as a white solid (8.1 mg, 20% from **4**). ^1H NMR ($CDCl_3$): δ 0.81 (s, 3H, 18-CH_3); 1.30-2.35 (m, 12H); 2.80-2.85 (m, 2H); 3.39 (s, 3H, -OCH_3); 3.64 (d, J = 5.4, 1H, 17-H);

3.68-3.74 (m, 1H, 16-H); 4.50-4.80 (b, < 1H, OH); 6.58-6.68 (m, 2H); 7.17 (d, J = 8.1, 1H).

16-Methoxy-estra-1,3,5(10)-triene-3,17-diol (6b, 6c). Methoxy triflate estrone (0.0694 mmol, 30 mg **9a** or 0.0176 mmol, 7.6 mg **9b**) was dissolved in freshly distilled Et_2O (0.013 mmol/mL), stirred under N_2, and cooled to -78 °C in a dry ice/isopropanol bath. A 1.0 M $LiAlH_4$ solution in Et_2O (0.350 mmol, 350 µL to **9a** or 0.087 mmol, 87 µL to **9b**) was added dropwise over ca. 2 m. The pale yellow reaction was stirred at -78 °C for 25 m after which it was removed from the cold bath and allowed to warm to rt over 25 m giving a cloudy white appearance. Addition of 6 N HCl (7.8 mmol, 1.3 mL for **9a** or 1.044 mmol, 0.174 mL for **9b**) quenched the reaction. The organic phase was removed and the remaining aqueous phase extracted with 1x3 mL Et_2O and 2x3 mL 1:1 CH_2Cl_2:hexane. Each organic extract was passed through a $MgSO_4$ plug (2 g) and a 0.22 µ filter. Solvent was removed under reduced pressure. Purification by semi-preparative normal phase HPLC (40% 1:19 isopropanol:CH_2Cl_2, 60% hexane) yielded **6b** (0.022 mmol, 6.6 mg, 31%) or **6c** (0.0175 mmol, 5.3 mg, 83%) as a white solid. **6b** ^{1}H NMR ($CDCl_3$): δ 0.71 (s, 3H, 18-CH_3); 1.20-2.40 (m, 12H); 2.78-2.85 (m, 2H); 3.40 (s, 3H, -OCH_3); 3.76 (d, J = 5.1, 1H, 17-H); 3.99-4.05 (m, 1H, 16-H); 4.68-4.80 (b, < 1H, OH); 6.55-6.68 (m, 2H); 7.16 (d, J = 8.4, 1H). HRMS calculated for $C_{19}H_{26}O_3$ (M^+) 302.1882, found 302.1883. **6c** ^{1}H NMR ($CDCl_3$): δ 0.79 (s, 3H, 18-CH_3); 0.95-2.40 (m, 12H); 2.80-2.85 (m, 2H); 3.37 (s, 3H, -OCH_3); 3.49 (d, J = 7.8, 1H, 17-H); 3.73-3.78 (m, 1H, 16-H); 6.55-6.65 (m, 2H); 7.16 (d, J = 8.7, 1H). HRMS calculated for $C_{19}H_{26}O_3$ (M^+) 302.1882, found 302.1881.

Time Line for Statement of Work

Goal	Year of Funding 0 1 2 3 4
1) Complete Required Course Work	----------> ....>complete
2) Synthesize Authentic Non-Radioactive Compounds*	----------> .....
3) Measure Binding Affinity of Non-Radioactive Compounds	----------> .....
4) Synthesize Radioactive Compounds	-------->
5) Evaluate Best Compounds in Animal Models	---------->
6) Write Thesis	-------->

----------> represents original time estimate
..........> represents work completed

*Three compounds have been synthesized to date.

List of Abbreviations

ACN	acetonitrile
BnBr	α-bromotoluene
CaH_2	calcium hydride
$CDCl_3$	deuterated chloroform: NMR solvent
$CFCl_3$	fluorotrichloromethane
CH_2Cl_2	methylene chloride
CH_3OF	methyl hypofluorite
CH_3OF•ACN	methyl hypofluorite/acetonitrile complex
$CHCl_3$	chloroform
COSY	correlation spectroscopy
ER	estrogen receptor
ER+	estrogen receptor positive
ES	estradiol: the natural ER ligand
Et_2O	diethyl ether
Et_3N	triethyl amine
EtOAc	ethyl acetate
EtOH	ethanol
F_2	fluorine (gas)
FES	[^{18}F]-16α-fluoroestradiol-17β
H_2	hydrogen (gas)
HCl	hydrochloric acid
HF	hydrofluoric acid
HMQC	heteronuclear multiple quantum coherence
HOAc	acetic acid
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrum

K_2CO_3	potassium carbonate
KF	potassium fluoride
$LiAlH_4$	lithium aluminum fluoride
MeOH	methanol
$MgSO_4$	magnesium sulfate
N_2	nitrogen (gas)
$Na_2S_2O_4$	sodium thiosulfate
$NaBH_4$	sodium borohydride
NaF	sodium fluoride
$NaHCO_3$	sodium bicarbonate
NaI	sodium iodide
NMR	nuclear magnetic resonance
NOESY	nuclear Overhauser effect spectroscopy
$PdCl_2$	palladium chloride
$PdCl_2(CH_3CH)_2$	palladium chloride diacetonitrile
PET	positron emission tomography
RBA	relative binding affinity
rt	room temperature
TEA	triethyl amine
Tf	trifluormethanesulfonyl (triflyl)
TLC	thin-layer chromatography
TMSCl	chlorotrimethyl silane
TMSOTf	trimethyl silyl triflate
TOCSY	total correlation spectroscopy
UV	ultraviolet

References

1. Braunschweiler, L.; Ernst, R. R. *J. Mag. Res.* **1983,** *53*, 521-528.

2. Cazeau, P.; Duboudin, F.; Moulines, F.; Babot, O.; Dunogues, J. *Tetrahedron* **1987,** *43*, 2075-2088.

3. Corey, E. J.; Knapp, S. *Tetrahedron Letters* **1976,** *51*, 4687-4690.

4. Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*, 5th ed.; John Wiley & Sons: New York, 1988.

5. Dehdashti, F.; Mortimer, J. E.; Siegel, B. A.; Griffeth, L. K.; Dobkin, J. A.; Bonasera, T. A.; Fusselman, M. J.; Detert, D. D.; Cutler, P. D.; Katzenellenbogen, J. A.; Welch, M. J. *Journal of Nuclear Medicine* **1995,** *36*, 1766-1774.

6. Katzenellenbogen, J. A. Estrogen and Progestin Radiopharmaceuticals for Imaging Breast Cancer. In *Estrogens, progestins, and their antagonists;* Pavlik, E. J. Ed.; Birkhäuser: Boston, 1996; Vol. 1; pp. 197-242.

7. Katzenellenbogen, J. A.; Jr., H. J. J.; Myers, H. N. *Biochemistry* **1973,** *12*, 4085-4092.

8. Kiesewetter, D. O.; Katzenellenbogen, J. A.; Kilbourn, M. R.; Welch, M. J. *Journal of Organic Chemistry* **1984,** *49*, 4900-4905.

9. Kol, M.; Rozen, S.; Appelman, E. *Journal of the American Chemical Society* **1991,** *113*, 2648-2651.

10. Långström, B. R. *Acta Chemica Scandinavia* **1990,** *147*, S374.

11. McCarthy, T. J.; Bonasera, T. A.; Welch, M. J.; Rozen, S. *J. Chem. Soc., Chemical Communications* **1993**, 561-562.

12. McGuire, A. H.; Dehdashti, F.; Siegel, B. A.; Lyss, A. P.; Brodack, J. W.; Mathias, C. J.; Mintun, M. A.; Katzenellenbogen, J. A.; Welch, M. J. *Journal of Nuclear Medicine* **1991,** *32*, 1526-1531.

13. Mintun, M. A.; Welch, M. J.; Siegel, B. A.; Mathias, C. J.; Brodack, J. W.; McGuire, A. H.; Katzenellenbogen, J. A. *Radiology* **1988,** *169*, 45-48.

14. Moriarty, R. M.; Prakash, O.; M.P.Duncan; Vaid, R. K. *J. Org. Chem* **1987,** *52*, 150-153.

15. Numazawa, M.; Nagaoka, M.; Tsuji, M. *J. Chem. Soc. Perkin Trans.* **1983,** *1*, 121-125.

16. Pomper, M. G.; VanBrocklin, H. F.; Thieme, A. M.; Thomas, R. D.; Kiesewetter, D. O.; Carlson, K. E.; Mathias, C. J.; Welch, M. J.; Katzenellenbogen, J. A. *Journal of Medicinal Chemistry* **1990,** *33*, 3143-3155.

17. Rozen, S.; Mishani, E.; Kol, M. *Journal of the American Chemical Society* **1992,** *114*, 7643-7645.

18. Vypel, H. *Chimia* **1985,** *39*, 305.